DNA Methylome Marks of Exposure to Particulate Matter at Three Time Points in Early Life

DNA Methylome Marks of Exposure to Particulate Matter at Three Time Points in Early Life

Michelle Plusquin, Marc Chadeau-Hyam, Akram Ghantous, Rossella Alfano, Mariona Bustamante, Leda Chatzi, Cyrille Cuenin, John Gulliver, Zdenko Herceg, Manolis Kogevinas, Tim S. Nawrot, Costanza Pizzi, Daniela Porta, Caroline L. Relton, Lorenzo Richiardi, Oliver Robinson, Jordi Sunyer, Roel Vermeulen, Annette Vriens, Martine Vrijheid, John Henderson, and Paolo Vineis[’ ’]

[-] Centre for Environmental Sciences, Hasselt University, Hasselt, Belgium
[-] Department of Epidemiology and Biostatistics, The School of Public Health, Imperial College London, London, United Kingdom
[-] Medical Research Council-Health Protection Agency Centre for Environment and Health, Imperial College London, London, United Kingdom
- Institute for Risk Assessment Sciences (IRAS), Division of Environmental Epidemiology, Utrecht University, Utrecht, The Netherlands
- International Agency for Research on Cancer (IARC), 150 Cours Albert-Thomas, 69008 Lyon, France
[-] Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain
- Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP), Madrid, Spain [-] ISGlobal, Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain
[-] Universitat Pompeu Fabra (UPF), Barcelona, Catalonia, Spain
[-] Department of Preventive Medicine, University of Southern California, Los Angeles, California 90007, United States
[-] Department of Social Medicine, University of Crete, Heraklion, Crete, Greece
[-] IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain
- Environment & Health Unit Leuven University, Leuven, Belgium
[-]Cancer Epidemiology Unit-CERMS, Department of Medical Sciences, University of Turin and CPO-Piemonte, Torino, Italy Department of Epidemiology of the Lazio Regional Health Service, Rome, Italy
[-] MRC Integrative Epidemiology Unit, Department of Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, U.K.
[-] Department of Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, U.K.
IIGM, Italian Institute for Genomic Medicine, Turin, Italy

* Supporting Information

ABSTRACT: Maternal exposure to airborne particulate matter (PM) has been associated with restricted fetal growth and reduced birthweight. Here, we performed methylome-wide analyses of cord and children’s blood DNA in relation to resi-dential exposure to PM smaller than 10 μm (PM_{10}). This study included participants of the Avon Longitudinal Study of Preg-nancy and Childhood (ALSPAC, cord blood, n = 780; blood at age 7, n = 757 and age 15−17, n = 850) and the EXPOsOMICS birth cohort consortium including cord blood from ENVIRON-AGE (n = 197), INMA (n = 84), Piccolipiù(n = 99) and Rhea (n = 75). We could not identify significant CpG sites, by meta-analyzing associations between maternal PM_{10} exposure during pregnancy and DNA methylation in cord blood, nor by studying DNA methylation and concordant annual exposure at 7 and 15−17 years. The CpG cg21785536 was inversely associated with PM_{10} exposure using a longitudinal model integrating the three studied age groups (−1.2% per 10 $\mu g/m^3$; raw p-value = 3.82×10^{-8}). Pathway analyses on the corresponding genes of the 100 strongest associated CpG sites of the longitudinal model revealed enriched pathways relating to the GABAergic synapse, p53 signaling and NOTCH1. We provided evidence that residential PM_{10} exposure in early life a□ects methylation of the CpG cg21785536 located on the EGF Domain Specific O-Linked N-Acetylglucosamine Transferase gene.

INTRODUCTION

Exposure to air pollution represents a major threat to health worldwide. Consequences are observed at all ages and even in areas with relatively low exposure levels.[1] The in utero envi-ronment can be perturbed by environmental exposures a□ecting developmental processes and thereby triggering adverse health e□ects later in life.[2] Several studies showed that maternal expo-sure to ambient air pollution and particulate matter (PM) was associated with restricted fetal growth or reduced birthweight.[3−6] During in utero development cells are epigenetically reprog-rammed making them particularly susceptible to environmental perturbations.[7] A few epigenome-wide studies investigated DNA methylation and exposure to air pollution, in adults[8−10] and in early life.[11−13] For example, prenatal exposure to ambient NO_2 was associated with the methylation status of DNA of mitochondria-related genes and genes involved in antioxidative defense pathways in cord blood.[9] A study comparing methylation profiles of blood samples of children (aged 7−15 years) from two regions with high and low air pollution reported di□erential meth-ylation at CpG sites in biological pathways including immune responses and DNA−protein binding.[13] An in vivo study on bronchial epithelial cells showed that diesel exhaust is associated with di□erential methylation of CpG sites located in genes with a role in transcription factor activity, protein metabolism, cell adhe-sion, and vascular development.[11] To understand the evolution of DNA methylation changes in early life associated with intra-uterine exposures, it is necessary to study longitudinal

measures of DNA methylation in relation to contemporaneous postnatal exposure. An in depth understanding of DNA methylation trajec-tories over time can distinguish causal DNA methylation changes from stochastic processes.

We first performed methylome-wide analyses in cord blood DNA in relation to maternal exposure during pregnancy to par-ticles smaller than 10 μm (PM_{10}) in independent population-based cohorts; the Avon Longitudinal Study of Parents and Chil-dren (ALSPAC)[14] and a consortium (EXPOsOMICS) of four birth cohorts: ENVIRONAGE, INMA, Rhea, Piccolipiu. In addition, we analyzed DNA methylation in serial venous blood samples from the same individuals at two additional time points (at age 7 and 15−17) in the ALSPAC cohort. Using a longitudinal model integrating DNA methylation of the three time points (i.e., birth, at 7 and 15−17 years of age) we aimed at identifying methylation signals in childhood in relation to prenatal and early life residential PM_{10} exposure (at 7 and 15−17 years of age).

MATERIALS AND METHODS

Participants. ALSPAC is a birth cohort study of children born in Avon, UK between April 1991 and December 1992.[14] In the framework of the Accessible Resource for Integrated Epigenomics Studies (ARIES) project (http://www.ariesepigenomics.org.uk/) DNA methylation data was collected. Mother-o□spring pairs (n = 1018) were selected based on the availability of DNA samples of the mother (prenatal and when the child(ren) were at age 17 years) and the children (at birth, at age 7 and at age 15−17 years).[21] Within the EXPOsOMICS collaborative European project,[15] a subset of four population-based birth cohorts, ENVIRONAGE,[16] INMA, Rhea and the Turin center of the Piccolipiu study, was established to conduct DNA methylation analyses. Sam-ples were selected randomly from each cohort (N = 200 for ENVIRONAGE, N = 99 for Piccolipiu,̀ N = 100 for INMA and N = 101 for Rhea) among participants with su□cient sample volume, quality and available covariate data. Detailed methods for each cohort are provided in the Supporting Information (SI) p 3.

Methylation Data. DNA methylation assays were per-formed at the University of Bristol, UK (ALSPAC) as part of the ARIES project,[21] at the International Agency for Research on Cancer, Lyon, France (ENVIRONAGE, Rhea, Piccolipiu)̀ and at the Genome Analysis Facility of the University Medical Center Groningen, The Netherlands (INMA). The Zymo EZ DNA meth-ylation kit (Zymo, Irvine, CA) was used for bisulphite-treatment of the DNA. DNA methylation was determined using the Illumina InfiniumVR HumanMethylation 450K BeadChip, arrays were scanned using an Illumina iScan and GenomeStudio software performed initial quality review.

ARIES data were preprocessed in R, with the me□l package. For the EXPOsOMICS data preprocessing we used in-house software written in the statistical software program R as described previously.[10]

The data was trimmed for outliers as defined by methylation levels which were more than three interquartile ranges below the first quartile or above the fourth quartile. Probes that target nonspecific CpG sites (n = 40 590),[22] showing a p-value for detection of >0.01, detected in less than 20% of the samples, or probes located on sex chromosomes (n = 11 648) were excluded. This left the following total of remaining probes:

i. in the ARIES study: 430 670, 430 699, and 430 710 probes in neonatal cord blood, peripheral blood in childhood and peripheral blood in adolescence, respectively.
ii. in the EXPOsOMICS study: 420 399, 387 965, 419 692, and 419 815 probes for ENVIRONAGE, INMA, Rhea, and Piccolipiu,̀ respectively.

The methylation levels of CpG loci were expressed as Beta values, calculated as the ratio of the intensity of methylated CpG loci and the sum of methylated and unmethylated CpG loci. CpGs were annotated to genes using the Illumina's genome coordinates[23] and UCSC Genome Browser.

Residential Air Pollution. In ALSPAC (including the ARIES sample), exposure to particulate matter ≤10 μm (PM_{10}) was modeled using dispersion modeling of annual average exposures for the period 1990−2008 based on daily total PM_{10} assessed at maternal residential addresses (including address changes). For pregnancy trimesters and infancy (birth to 6 months; 7 to 12 months) we used local (ADMS-Urban)[24] and regional/long-range (NAME-III)[25] air pollution models. For annual average expo-sures up to age 15, we assessed spatial contrasts in local

sources (ADMS-Urban) of PM_{10} with a yearly varying model constant for all background sources. We accounted for changes in address in all periods using a bespoke algorithm developed at Imperial College London: Algorithm for Generating Address-History and Exposures (ALGAE; https://smallareahealthstatisticsunit.github.io/algae/index.html).

In INMA, Rhea and Piccolipiùexposure to air pollutants was generated in the framework of the ESCAPE study. Following the ESCAPE protocol[26,27] the residential location of the mothers have been geocoded and particulate matter concentrations at the addresses of study participants were estimated by applying a standardized procedure of land-use regression models (LUR). Levels of PM with an aerodynamic diameter smaller than 10 μm were determined during di□erent seasons at residential sites for each study between October, 2008 and April, 2011.[26,27] LUR-models were established using the annual mean concentration as the dependent variable and an wide-ranging list of geographical variables as potential predictors.[26,27] These predictors were among others related to tra□c, population density, industry and altitude. A large portion of spatial variation was explained by the models, the R^2 from leave-one-out cross-validation was 0.87 for Spain (Barcelona), 0.53 for Greece (Heraklion) and 0.78 for Italy (Turin). Finally, the models were applied to determine exposure to PM_{10} at the mother's residential address. Details of this procedure are available via the following Web site: http://www. escapeproject.eu/manuals/. To temporally adjust the LUR PM_{10} levels to the pregnancy period, data from routine monitoring stations were used.

In ENVIRONAGE, the regional background levels of PM_{10} were modeled for each mother's residential address using a high resolution spatial temporal interpolation method that combines data from land cover obtained from satellite images (Corine land cover data set) with measures from monitoring stations (n = 34) and a dispersion model.[28] Validation statistics of the model showed that the spatial explained variance (R^2) for annual mean PM_{10} was more than 0.80.

Biosamples. In ARIES, a total of 1018 subjects with at least one available DNA methylation profile were selected (at birth: n = 914, 7 years: n = 980, 15 years: n = 981). Subjects that failed DNA methylation quality control (n = 53 for cord blood, n = 53 for childhood and n = 56 for adolescents) and those with missing values on confounders or residential PM_{10} levels (n = 81 for cord blood, n = 170 for childhood and n = 75 for adolescents) were removed. As a result, a total of 780, 757, and 850 subjects were included in the analyses for birth, childhood and adolescence, respectively.

For ENVIRONAGE, Rhea, Piccolipiu,out of the 399 analyzed samples, three failed quality control and 25 had missing values for confounders or exposures, leaving us with 371 subjects included for further analyses. In INMA only one sample failed and 15 did not have methylation data available, thus a total of 84 subjects were included in our analyses.

Statistical Analyses. As summarized in Figure 1 and SI Figure S1, we ran analyses looking at residential average PM_{10} exposure across (i) the entire pregnancy for cord blood samples, and (ii) for the year of blood sampling for childhood (7 years) and adolescence (15−17 years of age). In our epigenome-wide association studies, the analyses were conducted in three steps:

(i) in cord blood samples in relation to pregnancy exposure to PM_{10} (analyses were conducted for each study separately and resulting e□ect size estimates were subsequently meta-analyzed using fixed e□ects models with inverse-variance weighting);

(ii) at age at 7 in relation to concomitant annual PM_{10} exposure in the ARIES sample; (iii) at age 15−17 in relation to concom-itant annual PM_{10}, in ARIES.

In all these models, DNA methylation intensities at each CpG site were modeled as the dependent variable in a mixed linear model including microarray number and position on array as random e□ect and exposure as the explanatory variable. All mod-els were adjusted for sex of the child, maternal smoking during pregnancy, blood cell type proportions estimated using estab-lished deconvolution approaches for ARIES[29] and EXPOsO-MICS,[30] respectively.

We also investigated longitudinal e□ects of prenatal exposure on DNA methylation in early life in the ARIES sample, by first regressing pregnancy exposure to PM_{10} against DNA genome-wide DNA methylation at 7 or 15−17 years, using the same parametrization and confounding variables as described above, but additionally including annual PM_{10} exposure level at the corresponding year. Finally, adopting a multivariate normal model,

we integrated data (i.e., methylation profiles and exposure measurements) at the three time points, adjusting for the same set of confounding variables. We corrected for multiple testing by
controlling the False Discovery Rate (FDR) below 5% using a Benjamini-Hochberg procedure. Sensitivity analyses included additional adjustment on maternal age, gestational age (weeks), ethnicity (based on citizenship), maternal education (classified as obtained primary school, secondary school and higher education) and passive smoking at 7 years of age.

The 100 strongest associations, based on p-values, identified in the longitudinal analyses were further investigated using phen-ograms (chromosomal figure annotated with lines at specific base-pair locations - http://visualization.ritchielab.psu.edu/phenograms), and by performing an overrepresentation analysis (using DAVID software, https://david.ncifcrf.gov) of the associated genes. In this analysis enriched pathways with (i) enrichment p-values smaller than 0.05, (ii) fold changes larger than 1.5 and with (iii) at least three genes included were considered as significantly enriched.

In order to assess whether exposure to PM_{10} also a□ects meth-ylation sites associated with similar exposures, we performed look-up analyses based on (i) 6073 identified smoking CpG sites in a Pregnancy and Childhood epigenetics (PACE) study,[31]

(ii) 148 smoking related CpGs that met FDR significance at lookup replication level in older children in a PACE study[31] and
(iii)the 25 strongest associations CpG sites of a PACE study on maternal Nitrogen Oxide (NO_x) exposure.[12]

RESULTS

Table 1 depicts demographic characteristics and residential exposure to PM_{10} of the five participating cohorts: ALSPAC, ENVIRONAGE, INMA, Rhea and Piccolipiù.SI Figure S2 shows the distribution of PM_{10} exposure during pregnancy and SI Table S1 shows the correlation between PM_{10} exposure at birth, at 7 and 15 years of age.

1. Residential Prenatal and Early Life Exposure to PM_{10}.

The meta-analysis of the epigenome-wide association studies of PM_{10}-exposure during pregnancy in the five cohorts (n = 1235) did not show any CpG sites after correction for multiple testing using a threshold of 0.05 (Table 2 shows top 15 CpG sites). Furthermore, we observed heterogeneity between the five cohorts under study; 59% of the probes (n = 252,333) showed an $I^2 > 0.4$. In the ARIES sample, regressing the residential annual exposure to PM_{10} on methylation in childhood (at 7 years) and adolescence (15−17 years) did not reveal significant CpG sites after correction for multiple testing (SI Table S2).

Table 1. Characteristics of the Population[a]

	ALSPAC (n = 780)	ENVIRONAGE (n = 197)	INMA (n = 84)	Piccolipiù(n = 99)	Rhea (n = 75)
Maternal Characteristics					
maternal age ± SD	29.69 ± 4.39	29.29 ± 4.37	31.78 ± 4.04	33.33 ± 4.44	29.41 ± 4.86
maternal education					
(%) low	396 (51)	31 (16)	18 (22)	8 (8)	6 (8)
medium	230 (29)	71 (36)	38 (45)	41 (41)	42 (56)
high	154 (20)	95 (48)	28 (33)	50 (51)	27 (26)
maternal smoking (%)	107 (14)	24 (12)	10 (12)	8 (8)	10 (13)
parity >1 (%)	408 (53)	90 (46)	41 (49)	53 (54)	53 (71)
Newborn Characteristics					
girls (%)	403 (52)	96 (49)	44 (52)	45 (45)	36 (48)
birth weight (grams) ± SD	3491 ± 476	3390 ± 489	3316 ± 405	3221 ± 431	3253 ± 414
gestational age at delivery (weeks) ± SD	39.55 ± 1.49	39.10 ± 1.64	39.92 ± 1.59	39.50 ± 1.58	38.49 ± 1.37
In utero residential exposure to PM_{10} ($\mu g/m^3$) ± SD	20.58 ± 2.87	17.73 ± 2.38	46.69 ± 5.34	59.15 ± 14.18	35.54 ± 3.86
children's residential exposure to PM_{10}					
at childhood (7 years) ± SD	22.55 ± 1.04				
in adolescence (15−17 years) ± SD	23.97 ± 1.27				

[a]Information of variables are given as counts (percentages) or means ± standard deviation (SD).

2. Longitudinal Analysis. After correction for multiple testing in utero exposure to PM10 was not significantly associated with DNA methylation in childhood at 7 or 15−17 years of age (SI Tables S3 and S4), we then evaluated the persistence of the DNA methylation signals by investigating the ranking of the CpG sites based on p-values. Figure 2 shows that in the 7-year olds, results for three CpG sites (cg22377963 located in BCL7C gene, cg19718508 located in F12 gene, cg06406026) were among the 100 top ranked associations of both pregnancy and concurrent exposure to PM10 based on p-value, while in the 15−17 years-old sample only 1 CpG site was ranked in both top 100-lists (cg01363474 located in PPP2R2C gene). In addition, the CpG site cg25213055 located in EXOC2 gene was ranked at place 108
in relation to exposure of PM10 at 15−17 years.

To identify DNA methylation signals of longitudinal residen-tial PM10 exposure we integrated methylation profiles and expo-sure measurements at the three time points (during pregnancy, annual exposure at 7 and 15−17 years), and ran multivariate nor-mal models (Figure 3, SI Table S5). One CpG site (cg21785536) was identified as inversely associated with residential PM10 expo-sure over the 3 (during pregnancy, annual exposure at 7 and 15−17 years) studied age groups (β=-0.0012; raw p-value = 3.82 × 10−8, q-value: 0.016). Sensitivity analyses additionally adjusting for respectively maternal age, gestational age, ethnicity, maternal education and environmental tobacco smoking at 7 years of age did not a□ect the direction of this association and slightly attenuated the p-value (p-values ranged from 3.44 × 10−8 to 6.04 × 10−8, SI Figure S3). As illustrated by Figure 4, the 100 strongest associated CpG loci are spread across all chromosomes except chromosome 21.

Table 2. Study on cord blood DNA methylation and PM_{10} exposure during pregnancy. Results from the inverse weighted meta-analysis[a]

CpG	CHR	gene	localization on gene	localization on CGI	β	SE	p-value	direction	I^2
cg27026202	2:235200560				−0.740	0.198	1.91×10^{-4}	-----	11.9
cg20380368	15:70342278	TLE3	3UTR		0.750	0.203	2.24×10^{-4}	++---	88.0
cg02326285	14:79111719	NRXN3	5UTR		0.826	0.224	2.30×10^{-4}	-++--	76.0
cg21341928	13:25670327	PABPC3	1stExon	Island	−1.285	0.350	2.45×10^{-4}	++---	57.3
cg18136930	7:4831038	KIAA0415	3UTR	North Shore	0.736	0.201	2.50×10^{-4}	++-+-	58.2
cg16847315	7:116139414	CAV2	TSS200	North Shore	−0.661	0.181	2.51×10^{-4}	-+-+-	37.2
cg25846290	7:144100799	NOBOX	TSS200		0.729	0.199	2.56×10^{-4}	-----	77.8
cg09582545	22:39440294	APOBEC3F	3UTR		0.918	0.251	2.61×10^{-4}	---++	9.19
cg20098659	12:10183364	CLEC9A	1stExon		−1.230	0.337	2.62×10^{-4}	+-+++	2.97
cg03950476	1:53019814	ZCCHC11	TSS1500	South Shore	−0.732	0.201	2.64×10^{-4}	----+	49.0
cg13881452	6:169601968				0.684	0.188	2.66×10^{-4}	++---	50.0
cg01680054	3:491979				−0.535	0.147	2.79×10^{-4}	-++--	83.2
cg14371343	7:122097744	CADPS2	Body		−0.490	0.135	2.82×10^{-4}	-++--	79.9
cg22393213	2:237478526	CXCR7	1stExon	South Shore	−0.551	0.152	2.91×10^{-4}	++---	69.2
cg16034268	8:41188196				−0.610	0.169	2.98×10^{-4}	-----	49.2

[a]Top 15 CpG sites based on p-values from the epigenome-wide association study of exposure to PM_{10} during pregnancy, n = 1235. β (regression coe□cient) represents the di□erence in methylation for 10 units (μg/m^3) increase of PM_{10}. Adjusted p-values (correction for multiple testing) were equal to 1 for all the CpGs and thus are not shown in the table. The transcription start site and untranslated region are abbreviated as respectively TSS and UTR. The column headers stand for: UCSC annotated gene (gene), chromosome and chromosomal position (CHR), UCSC gene region feature category (localization on gene); UCSC relation to CpG islands (Localization on CGI); regression coe□cient (β); standard error for regression coe□cient (SE); direction of the associations for each cohort (Rhea, INMA, Piccolipiu, ENVIRONAGE, ALSPAC, respectively); I squared heterogeneity statistic (I^2). Models were adjusted for technical variables, sex of the child, maternal smoking during pregnancy, and estimated blood cell composition.

Insight into the underlying mechanisms of the top 100 CpG sites was pursued by gene enrichment analyses based on its cor-responding gene list (n = 75) (Table 3). We identified five enriched pathways. One relates to the GABAergic synapse that plays a role in neurotransmission, one to p53 signaling that plays a role in tumor suppression and three to NOTCH1, a sig-naling pathway. SI Figure S4 shows volcano plots of associations in the longitudinal model restricted to the CpG sites located on genes involved in these three pathways. The plots show an enrichment of negative associations which suggests that exposure leads more often to lower methylation of the CpG sites in these pathways.

3. Lookup Analyses. Employing a CpG look-up approach to the longitudinal model, we explored whether exposure to PM_{10} also a□ects methylation sites associated with similar exposures. Among three sets of selected CpG sites identified as related to maternal smoking or to prenatal NO_x-exposure,[12,31] we did not identify significant associations to PM_{10}-exposure after FDR correction (number of comparisons = 6073 and 148 for the two smoking sets and 25 for NO_x exposure set) (SI Figure S5). However, the CpG sites cg07571337 (β = 0.0020, raw p-value = 2.84×10^{-3}), cg26500033 (β = 0.0023, raw p-value = 6.53×10^{-3}) and cg10704395 (β = −0.0020, raw p-value = 8.12×10^{-3}), present in the set of maternal smoking signals that are persistent in early childhood[31] were associated with the same direction to PM_{10} with an adjusted p-value of 0.24.

DISCUSSION

Evidence of prenatal exposure to air pollution a□ecting the cord blood or placenta methylome is available from a few published studies.[12,32−36] By collecting blood samples at multiple time points through the early life course, our study o□ers the first evi-dence that exposure to particulate matter at birth and throughout early life induces epigenetic alterations in children and adoles-cents. Contemporaneous residential PM exposure as well as longitudinal modeling of the three time points in early life were moderately associated with single DNA methylation entities. The identified targets from birth to adolescence might point toward neurological and cell division control mechanisms.

In our study, one CpG site (cg21785536) located in the region before the transcription start site of EOGT, the eukaryotic growth factor (EGF) Domain Specific O-Linked N-Acetylglucosamine transferase (previously C3orf64) met strict FDR-corrected statis-tical significance. This enzyme modifies proteins containing EGF-like domains, and is employed in regulating the NOTCH receptor signaling pathways.[37] This observation is further strength-ened by the result of our overrepresentation pathway analysis showing that the NOTCH1-pathway is associated with PM_{10} across childhood. The p53-pathway, important in cell-cycle checkpoint control, was additionally identified in this overrepre-sentation analyses. Both are commonly dysregulated pathways in cancer development.[38,39] Moreover, in relation to pregnancy exposure of PM_{10}, our analyses provided a potential epigenetic downregulated CpG site located in BCL7C, a gene involved in tumor suppression that may be also relevant to exposure in early childhood (7 years). These observations are suggestive evidence of an involvement of cell-cycle control underlying e□ects of exposure to air pollution. In regard of the young age of the present study population, these results may be interpreted within the context of the developmental origin of disease (DOHAD) hypothesis and reflect biomolecular changes exhibiting possible health e□ects later in life. The findings can also be relevant in the context of developmental processes, such as for example for EOGT that plays a role in ligand-induced NOTCH signaling required in endothelial cells for optimal vascular development,[40] and p53 that regulates di□erentiation and the response of embry-onic cells to diverse environmental stresses.[41] Our observations are in concordance with a study reporting di□erential methylation of the tumor suppressor genes: APC, p16, p53, and RASSF1A, in peripheral blood of steel workers with a well-characterized exposure to PM, after working 4 days compared to the baseline (before working).[42]

As the brain neocortex rapidly develops, pregnancy or infancy are sensitive periods for exposure to pollutants, this is exemplified by studies that show that exposure to air pollutants in early life are related to cognitive delays.[43−45] A notable finding of our study is a possible involvement of the GABAergic response, a candidate mechanism possibly supporting the biological plau-sibility of the disease pathogenesis. Four CpG sites located on genes that are among the 100 strongest longitudinal associations are involved in this pathway: SLC6A1, CACNA1C, GAD1, GABARAP. The CpG cg20837354 is correlated in blood and brain in the entorhinal cortex.[46] GABAergic neurons are a highly heteroge-neous group of cells[47] that are critical for the development of the neocortex. Alterations in GABAergic actions have been causally linked to developmental brain disorder[48] and GABAergic signal-ing is dysregulated in aging (reviewed in[49]). Our observations are also reinforced by a study on BDNF expression that was decreased in placental tissue with increasing in utero exposure to $PM_{2.5}$,[50] BDNF in turn modulates the GABA transporter GAT1.[51]

Based on lookup analyses, we could not vigorously observe biologic mechanisms shared between PM and other similar expo-sures, such as maternal tobacco smoking[31] and NO_x exposure.[12] Although mechanisms that have been related to smoking and air pollution exposure may a□ect similar health outcomes, we could not prove that the same epigenetic signals are targeted in early life. A possible explanation might be that particulate matter expo-sure occurs at levels that are much lower than smoking and fine particles have a specific toxicity.[52]

Of particulate note is the higher commonality between di□er-ential methylation associated with pregnancy exposure in child-hood (at 7 years of age) than in adolescence (15−17 years) in the present study. While the dynamics and stability of methylation markers over time are not well understood,[53−59] previous studies demonstrated that intraindividual variability of methylome during the first two years of life is mainly located within genes with important biological functions including immunity and

Table 3. Pathways Associated with the top 100 CpG-Sites Based on P-Values from the Longitudinal Model

database	term	count	p-value	fold enrichment
KEGG_PATHWAY	hsa04727: GABAergic synapse	4	0.0030	13.01
REACTOME_PATHWAY	R-HSA-2122947:R-HSA-2122947: NOTCH1 intracellular domain regulates transcription	3	0.0133	16.55
REACTOME_PATHWAY	R-HSA-2894862:R-HSA-2894862: constitutive signaling by NOTCH1 HD+PEST domain mutants	3	0.0191	13.65
REACTOME_PATHWAY	R-HSA-2644606:R-HSA-2644606: constitutive signaling by NOTCH1 PEST domain mutants	3	0.0191	13.65
KEGG_PATHWAY	hsa04115: p53 signaling pathway	3	0.0223	12.38

inflammation.[60,61] Air pollutants may induce systemic oxidative stress, as well as inflammation, changes in blood coagulation, endothelial function, and hemodynamic responses.[62,63] In 7-year-olds, a CpG site located on the gene body of the coagulation factor F12 was inversely associated with pregnancy exposure to PM_{10}, independent of their exposure at 7 years. In an experimental study, Killinc and colleagues showed that coarse PM promotes a long lasting thrombogenic e□ect predominantly via formation of activated F12.[64] Early life methylation modifications might have an influence on later life thrombotic susceptibility to PM. PM exposures may also lead to changes in hemoglobin, platelets, and white blood cells,[65] which may potentially contribute to the association between PM and adverse fetal growth.[66]

The epigenome-wide study in cord blood of Gruzieva and colleagues[12] identified 3 CpG sites in mitochondria-related genes associated with maternal NO_2 exposure during pregnancy. In their study, one CpG site was also found significant in older children. In spite of the reasonable size of our epigenome-wide study in cord blood and variability in exposures, the heterogeneity between five cohorts inevitably attenuates sensitivity. Some of the associations we identified with PM_{10} showed large heterogeneity across studies (59% with $I^2 > 40\%$). This suggests that, of the significant di□er-ences in methylation we observed in the meta-analyses, some may not be detected in all studies, and may therefore be driven by study-specific exposure patterns and/or study-related factors.

We report very small-magnitude e□ect sizes resulting from PM_{10}-exposure, which is a common finding in environmental epi-genetic studies and could be due to methylation di□erences in fraction of cells.[67] In the ALSPAC study, we did not have specific information about fractions of PM smaller than PM_{10}. Eeftens and colleagues reported high correlations of the spatial variation within areas between $PM_{2.5}$ and PM_{10} in the ESCAPE study that includes the same regions as INMA (Barcelona, Spain), Rhea (Heraklion, Greece), and Piccolipiù(Italy).[68] Exposure models of ALSPAC (dispersion model) and ENVIRONAGE (combina-tion of dispersion model and land use regression - LUR) di□er in method from the ESCAPE model (LUR), which might explain part of the heterogeneity in the present study. We combined five cohorts to study cord blood methylation, but we could not rep-licate our longitudinal findings in an independent cohort due to the unique nature of the ARIES data set.

Our study has specific limitations and strengths. Using over-representation analyses, we report several pathways possibly involved in the epigenetic response to air pollution. However, these functional hypotheses would require validation in further studies, for example using established biomarkers relating to these candidate pathways. The study of DNA methylation in (cord) blood or tissue presents a particular challenge as aggregate DNA methylation measures reflect a mixture of di□erent cell types. To overcome this problem, we adjusted the epigenome-wide studies for cell composition using established prediction models to infer blood cell composition both for cord and peripheral blood. However, these estimated cell count may not fully accurately represent actual blood cell composition and upon adjustment our study could still su□er from residual confounding. Refined modeling of tissue heterogeneity would rely on analyses carried out on purified cell or on single-cell analysis. Since cord blood and adult peripheral blood reflect di□erent physiological states and comprise cells with di□erent morphology, maturity, and functions, di□erences between the two matrices could not be taken into account in our longitudinal study.

The combination of a cord blood methylome study on indi-vidual air pollution exposure in five European birth cohorts and a longitudinal study of air pollution provided by serial samples in the ARIES study, is a major strength of this study. We provided a comprehensive study on methylation changes in relation to air pollution across childhood.

Using longitudinal measurements of DNA methylation, we have provided some evidence that PM_{10} exposure is associated with blood DNA methylation. From our agnostic approach, sev-eral novel CpG sites and mechanisms, that may create a molec-ular basis for the association between air pollution and health outcomes, have been detected. The identified targets might point toward neurological, cell division control and coagulation mech-anisms, though they have to be further tested in future studies.

ASSOCIATED CONTENT

*S Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b06447.

Figure S1: Flowchart of the study Figure S2: Violin plot of exposure to PM_{10} during pregnancy in the five participating cohorts Figure S3: Sensitivity analyses of CpG site cg21785536 Figure S4: Volcano plots of gene sets of the P53 signaling pathway and the GABAergic synapse pathway. Figure S5: Volcano plots of gene sets of maternal smoking and prenatal NO_x exposure. Table S1: Pearson correlation between PM_{10} exposure at birth, at 7 and 15 years of age. Table S2: Association of the cross-sectional study on DNA methylation and PM_{10} exposure during childhood (7 years of age) and adolescence (15−17 years of age). Table S3: List of 100 strongest association of the epigenome-wide association study in children of 7 years old and PM_{10} exposure during preg-nancy and corrected for the corresponding year of sampling. Table S4: List of 100 strongest association of the epigenome-wide association study in children of 15−17 years old and PM_{10} exposure during pregnancy and corrected for the corresponding year of sampling. Table S5:100 strongest association of the longitudinal study on DNA methylation and PM_{10} exposure during pregnancy, childhood (7 years of age) and adolescence (15−17 years of age)(PDF)

AUTHOR INFORMATION

Corresponding Author
*E-mail: p.vinies@ic.ac.uk.

Author Contributions
%Joint first authors

Funding

This work was carried out within the scope of the European Commission seventh Framework program grant EXPOsOMICS (308610-FP7 to PV). M.P. received support for the was supported by the European Commission seventh Framework program Marie Curie Actions grant (628858). The Centre for Environment and Health is supported by the Medical Research Council and Public Health England (MR/L01341X/1). M.C.-H. acknowledges support from the “Mechanomics” Population Research Committee project (22184). The ENVIRONAGE birth cohort is supported by grants from the European Research Council (ERC-2012-StG310898) and the Flemish Scientific Fund (FWO, 1516112N/G.0873.11.N.10). ARIES was funded by the BBSRC (BBI025751/1 and BB/I025263/1). The ALSPAC study was core-supported by the Medical Research Council, the Wellcome Trust (Grant ref: 102215/2/13/1), and the University of Bristol. Financial support for the generation of DNA methylation data within the ARIES study was provided by MRC, National Institutes for Health. ARIES is maintained and hosted within the MRC Integrative Epidemiology Unit at the University of Bristol (MC_UU_12013/2 and MC_UU_12013/8). Piccolipiùcohort funded by the Italian National Centre for Disease Prevention and Control (CCM grant 2010) and by the Italian Ministry of Health (art 12 and 12bis Dl.gs.vo 502/92). A.G. acknowledges support from Plan Cancer-Eva-Inserm research grant. Piccolipiùcohort acknowledges Andrea Ranzi for his contribution to the assessment of exposure to air pollution.

The authors declare no competing financial interest.

REFERENCES

(1) Cohen, A. J.; Brauer, M.; Burnett, R.; Anderson, H. R.; Frostad, J.; Estep, K.; Balakrishnan, K.; Brunekreef, B.; Dandona, L.; Dandona, R.; Feigin, V.; Freedman, G.; Hubbell, B.; Jobling, A.; Kan, H.; Knibbs, L.; Liu, Y.; Martin, R.; Morawska, L.; Pope, C. A.,

3rd; Shin, H.; Straif, K.; Shaddick, G.; Thomas, M.; van Dingenen, R.; van Donkelaar, A.; Vos, T.; Murray, C. J. L.; Forouzanfar, M. H. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. Lancet 2017, 389 (10082), 1907−1918.

(2) Barker, D. J. The fetal and infant origins of adult disease. BMJ. (Clinical research ed.) 1990, 301 (6761), 1111.

(3) Dadvand, P.; Ostro, B.; Figueras, F.; Foraster, M.; Basagana, X.; Valentin, A.; Martinez, D.; Beelen, R.; Cirach, M.; Hoek, G.; Jerrett, M.; Brunekreef, B.; Nieuwenhuijsen, M. J. Residential proximity to major roads and term low birth weight: the roles of air pollution, heat, noise, and road-adjacent trees. Epidemiology 2014, 25 (4), 518−25.

(4) Lamichhane, D. K.; Leem, J. H.; Lee, J. Y.; Kim, H. C. A meta-analysis of exposure to particulate matter and adverse birth outcomes. Environ. Health Toxicol 2015, 30, e2015011.

(5) Pedersen, M.; Giorgis-Allemand, L.; Bernard, C.; Aguilera, I.; Andersen, A. M.; Ballester, F.; Beelen, R. M.; Chatzi, L.; Cirach, M.; Danileviciute, A.; Dedele, A.; Eijsden, M.; Estarlich, M.; Fernandez-Somoano, A.; Fernandez, M. F.; Forastiere, F.; Gehring, U.; Grazuleviciene, R.; Gruzieva, O.; Heude, B.; Hoek, G.; de Hoogh, K.; van den Hooven, E. H.; Haberg, S. E.; Jaddoe, V. W.; Klumper, C.; Korek, M.; Kramer, U.; Lerchundi, A.; Lepeule, J.; Nafstad, P.; Nystad, W.; Patelarou, E.; Porta, D.; Postma, D.; Raaschou-Nielsen, O.; Rudnai, P.; Sunyer, J.; Stephanou, E.; Sorensen, M.; Thiering, E.; Tuffnell, D.; Varro, M. J.; Vrijkotte, T. G.; Wijga, A.; Wilhelm, M.; Wright, J.; Nieuwenhuijsen, M. J.; Pershagen, G.; Brunekreef, B.; Kogevinas, M.; Slama, R. Ambient air pollution and low birthweight: a European cohort study (ESCAPE). Lancet Respir. Med. 2013, 1 (9), 695−704.

(6) Schembari, A.; de Hoogh, K.; Pedersen, M.; Dadvand, P.; Martinez, D.; Hoek, G.; Petherick, E. S.; Wright, J.; Nieuwenhuijsen, M. J. Ambient Air Pollution and Newborn Size and Adiposity at Birth: Differences by Maternal Ethnicity (the Born in Bradford Study Cohort). Environ. Health Perspect 2015, 123 (11), 1208−15.

(7) Nafee, T. M.; Farrell, W. E.; Carroll, W. D.; Fryer, A. A.; Ismail, K. M. Epigenetic control of fetal gene expression. BJOG 2008, 115 (2), 158−68.

(8) Ward-Caviness, C. K.; Nwanaji-Enwerem, J. C.; Wolf, K.; Wahl, S.; Colicino, E.; Trevisi, L.; Kloog, I.; Just, A. C.; Vokonas, P.; Cyrys, J.; Gieger, C.; Schwartz, J.; Baccarelli, A. A.; Schneider, A.; Peters, A. Long-term exposure to air pollution is associated with biological aging. Oncotarget 2016, 7 (46), 74510−74525.

(9) Panni, T.; Mehta, A. J.; Schwartz, J. D.; Baccarelli, A. A.; Just, A. C.; Wolf, K.; Wahl, S.; Cyrys, J.; Kunze, S.; Strauch, K.; Waldenberger, M.;Peters, A. Genome-Wide Analysis of DNA Methylation and Fine Particulate Matter Air Pollution in Three Study Populations: KORA F3, KORA F4, and the Normative Aging Study. Environ. Health Perspect 2016, 124 (7), 983−90.

(10) Plusquin, M.; Guida, F.; Polidoro, S.; Vermeulen, R.; Raaschou-Nielsen, O.; Campanella, G.; Hoek, G.; Kyrtopoulos, S. A.; Georgiadis, P.; Naccarati, A.; Sacerdote, C.; Krogh, V.; Bas Bueno-de-Mesquita, H.; Monique Verschuren, W. M.; Sayols-Baixeras, S.; Panni, T.; Peters, A.; Hebels, D.; Kleinjans, J.; Vineis, P.; Chadeau-Hyam, M. DNA methylation and exposure to ambient air pollution in two prospective cohorts. Environ. Int. 2017, 108, 127−136.

(11) Clifford, R. L.; Jones, M. J.; MacIsaac, J. L.; McEwen, L. M.; Goodman, S. J.; Mostafavi, S.; Kobor, M. S.; Carlsten, C. Inhalation of diesel exhaust and allergen alters human bronchial epithelium DNA methylation. J. Allergy Clin. Immunol. 2017, 139 (1), 112−121.

(12) Gruzieva, O.; Xu, C. J.; Breton, C. V.; Annesi-Maesano, I.; Anto, J. M.; Auffray, C.; Ballereau, S.; Bellander, T.; Bousquet, J.; Bustamante, M.; Charles, M. A.; de Kluizenaar, Y.; den Dekker, H. T.; Duijts, L.; Felix, J. F.; Gehring, U.; Guxens, M.; Jaddoe, V. V.; Jankipersadsing, S. A.; Merid, S. K.; Kere, J.; Kumar, A.; Lemonnier, N.; Lepeule, J.; Nystad, W.; Page, C. M.; Panasevich, S.; Postma, D.; Slama, R.; Sunyer, J.; Soderhall, C.; Yao, J.; London, S. J.; Pershagen, G.; Koppelman, G. H.; Melen, E. Epigenome-Wide Meta-Analysis of Methylation in Children Related to Prenatal NO2 Air Pollution Exposure. Environ. Health Perspect 2017, 125 (1), 104−110.

(13) Rossnerova, A.; Tulupova, E.; Tabashidze, N.; Schmuczerova, J.; Dostal, M.; Rossner, P., Jr.; Gmuender, H.; Sram, R. J. Factors affecting the 27K DNA methylation pattern in asthmatic and healthy children from locations with various environments. Mutat. Res., Fundam. Mol. Mech. Mutagen. 2013, 741−742, 18−26.

(14) Fraser, A.; Macdonald-Wallis, C.; Tilling, K.; Boyd, A.; Golding, J.; Davey Smith, G.; Henderson, J.; Macleod, J.; Molloy, L.; Ness, A.; Ring, S.; Nelson, S. M.; Lawlor, D. A. Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. International journal of epidemiology 2013, 42 (1), 97−110.

(15) Vineis, P.; Chadeau-Hyam, M.; Gmuender, H.; Gulliver, J.; Herceg, Z.; Kleinjans, J.; Kogevinas, M.; Kyrtopoulos, S.; Nieuwenhuijsen, M.; Phillips, D. H.; Probst-Hensch, N.; Scalbert, A.; Vermeulen, R.; Wild, C. P.; Consortium, E. X. The exposome in practice: Design of the EXPOsOMICS project. Int. J. Hyg. Environ. Health 2017, 220 (2 Pt A), 142−151.

(16) Janssen, B. G.; Madhloum, N.; Gyselaers, W.; Bijnens, E.; Clemente, D. B.; Cox, B.; Hogervorst, J.; Luyten, L.; Martens, D. S.; Peusens, M.; Plusquin, M.; Provost, E. B.; Roels, H. A.; Saenen, N. D.; Tsamou, M.; Vriens, A.; Winckelmans, E.; Vrijens, K.; Nawrot, T. S. Cohort Profile: The ENVIRonmental influence ON early AGEing (ENVIRONAGE): a birth cohort study. International journal of epidemiology 2017, 46 (5), 1386−1387m.

(17) Guxens, M.; Ballester, F.; Espada, M.; Fernandez, M. F.; Grimalt, J. O.; Ibarluzea, J.; Olea, N.; Rebagliato, M.; Tardon, A.; Torrent, M.; Vioque, J.; Vrijheid, M.; Sunyer, J.; Project, I. Cohort Profile: the INMA–INfancia y Medio Ambiente–(Environment and Childhood) Project. International journal of epidemiology 2012, 41 (4), 930−40.

(18) Chatzi, L.; Leventakou, V.; Vafeiadi, M.; Koutra, K.; Roumeliotaki, T.; Chalkiadaki, G.; Karachaliou, M.; Daraki, V.; Kyriklaki, A.; Kampouri, M.; Fthenou, E.; Sarri, K.; Vassilaki, M.; Fasoulaki, M.; Bitsios, P.; Koutis, A.; Stephanou, E. G.; Kogevinas, M. Cohort Profile: The Mother-Child Cohort in Crete, Greece (Rhea Study). International journal of epidemiology 2017, 46 (5), 1392−1393k.

(19) Chatzi, L.; Plana, E.; Daraki, V.; Karakosta, P.; Alegkakis, D.; Tsatsanis, C.; Kafatos, A.; Koutis, A.; Kogevinas, M. Metabolic syndrome in early pregnancy and risk of preterm birth. Am. J. Epidemiol. 2009, 170 (7), 829−36.

(20) Farchi, S.; Forastiere, F.; Vecchi Brumatti, L.; Alviti, S.; Arnofi, A.; Bernardini, T.; Bin, M.; Brescianini, S.; Colelli, V.; Cotichini, R.; Culasso, M.; De Bartolo, P.; Felice, L.; Fiano, V.; Fioritto, A.; Frizzi, A.; Gagliardi, L.; Giorgi, G.; Grasso, C.; La Rosa, F.; Loganes, C.; Lorusso, P.; Martini, V.; Merletti, F.; Medda, E.; Montelatici, V.; Mugelli, I.; Narduzzi, S.; Nistico, L.; Penna, L.; Piscianz, E.; Piscicelli, C.; Poggesi, G.; Porta, D.; Ranieli, A.; Rapisardi, G.; Rasulo, A.; Richiardi, L.; Rusconi, F.; Serino, L.; Stazi, M. A.; Toccaceli, V.; Todros, T.; Tognin, V.; Trevisan, M.; Valencic, E.; Volpi, P.; Ziroli, V.; Ronfani, L.; Di Lallo, D. Piccolipiu, a multicenter birth cohort in Italy: protocol of the study. BMC Pediatr. 2014, 14, 36.

(21) Relton, C. L.; Gaunt, T.; McArdle, W.; Ho, K.; Duggirala, A.; Shihab, H.; Woodward, G.; Lyttleton, O.; Evans, D. M.; Reik, W.; Paul, Y. L.; Ficz, G.; Ozanne, S. E.; Wipat, A.; Flanagan, K.; Lister, A.; Heijmans, B. T.; Ring, S. M.; Davey Smith, G. Data Resource Profile: Accessible Resource for Integrated Epigenomic Studies (ARIES).
International journal of epidemiology 2015, 44 (4), 1181−90.
(22) Price, M. E.; Cotton, A. M.; Lam, L. L.; Farre, P.; Emberly, E.; Brown, C. J.; Robinson, W. P.; Kobor, M. S. Additional annotation enhances potential for biologically-relevant analysis of the Illumina Infinium HumanMethylation450 BeadChip array. Epigenet. Chromatin 2013, 6 (1), 4.
(23) Infinium HumanMethylation450K v1.2 Product Files. http:// support.illumina.com/downloads/infinium_humanmethylation450_ product_files.html.
(24) Carruthers, D. J.; Holroy, D. R. J.; Hunt, J. C. R.; Weng, W. S.; Robins, A. G.; Apsley, D. D.; Thomson, D. J.; Smith, F. B. Uk-Adms - a New Approach to Modeling Dispersion in the Earths Atmospheric Boundary-Layer. J. Wind Eng. Ind. Aerod 1994, 52 (1−3), 139−153.
(25) Jones, A.; Thomson, D.; Hort, M.; Ben. The UK Met Office's next-generation atmospheric dispersion model, NAME III. Nato-Chal M 2007, 17, 580.
(26) Eeftens, M.; Beelen, R.; de Hoogh, K.; Bellander, T.; Cesaroni, G.; Cirach, M.; Declercq, C.; Dedele, A.; Dons, E.; de Nazelle, A.; Dimakopoulou, K.; Eriksen, K.; Falq, G.; Fischer, P.; Galassi, C.; Grazuleviciene, R.; Heinrich, J.; Hoffmann, B.; Jerrett, M.; Keidel, D.; Korek, M.; Lanki, T.; Lindley, S.; Madsen, C.; Molter, A.; Nador, G.; Nieuwenhuijsen, M.; Nonnemacher, M.; Pedeli, X.; Raaschou-Nielsen, O.; Patelarou, E.; Quass, U.; Ranzi, A.; Schindler, C.; Stempfelet, M.; Stephanou, E.; Sugiri, D.; Tsai, M. Y.; Yli-Tuomi, T.; Varro, M. J.; Vienneau, D.; Klot, S.; Wolf, K.; Brunekreef, B.; Hoek, G. Development of Land Use Regression models for PM(2.5), PM(2.5) absorbance, PM(10) and PM(coarse) in 20 European study areas; results of the ESCAPE project. Environ. Sci. Technol. 2012, 46 (20), 11195−205.
(27) Beelen, R.; Hoek, G.; Vienneau, D.; Eeftens, M.; Dimakopoulou, K.; Pedeli, X.; Tsai, M. Y.; Kunzli, N.; Schikowski, T.; Marcon, A.; Eriksen, K. T.; Raaschou-Nielsen, O.; Stephanou, E.; Patelarou, E.; Lanki, T.; Yli-Toumi, T.; Declercq, C.; Falq, G.; Stempfelet, M.; Birk, M.; Cyrys, J.; von Klot, S.; Nador, G.; Varro, M. J.; Dedele, A.; Grazuleviciene, R.; Molter, A.; Lindley, S.; Madsen, C.; Cesaroni, G.; Ranzi, A.; Badaloni, C.; Hoffmann, B.; Nonnemacher, M.; Kraemer, U.; Kuhlbusch, T.; Cirach, M.; de Nazelle, A.; Nieuwenhuijsen, M.; Bellander, T.; Korek, M.; Olsson, D.; Stromgren, M.; Dons, E.; Jerrett, M.; Fischer, P.; Wang, M.; Brunekreef, B.; de Hoogh, K. Development of NO2 and NOx land use regression models for estimating air pollution exposure in 36 study areas in Europe - The ESCAPE project. Atmos. Environ. 2013, 72, 10−23.
(28) Maiheu, B. V. B.; Viaene, P.; De Ridder, K.; Lauwaet, D.; Smeets, N.; Deutsch, F.; Janssen, S. Identifying the Best Available Large-Scale Concentration Maps for Air Quality in Belgium. Study Commissioned by the Flemish Environment (MIRA); Flemish Institute for Technological Research (VITO): Mol, Belgium, 2013 [in Dutch].
(29) Gervin, K.; Page, C. M.; Aass, H. C.; Jansen, M. A.; Fjeldstad, H. E.; Andreassen, B. K.; Duijts, L.; van Meurs, J. B.; van Zelm, M. C.; Jaddoe, V. W.; Nordeng, H.; Knudsen, G. P.; Magnus, P.; Nystad, W.; Staff, A. C.; Felix, J. F.; Lyle, R. Cell type specific DNA methylation in cord blood: A 450K-reference data set and cell count-based validation of estimated cell type composition. Epigenetics 2016, 11 (9), 690−698.
(30) Bakulski, K. M.; Feinberg, J. I.; Andrews, S. V.; Yang, J.; Brown, S.; S, L. M.; Witter, F.; Walston, J.; Feinberg, A. P.; Fallin, M. D. DNA methylation of cord blood cell types: Applications for mixed cell birth studies. Epigenetics 2016, 11 (5), 354−62.
(31) Joubert, B. R.; Felix, J. F.; Yousefi, P.; Bakulski, K. M.; Just, A. C.; Breton, C.; Reese, S. E.; Markunas, C. A.; Richmond, R. C.; Xu, C. J.; Kupers, L. K.; Oh, S. S.; Hoyo, C.; Gruzieva, O.; Soderhall, C.; Salas, L.A.; Baiz, N.; Zhang, H.; Lepeule, J.; Ruiz, C.; Ligthart, S.; Wang, T.; Taylor, J. A.; Duijts, L.; Sharp, G. C.; Jankipersadsing, S. A.; Nilsen, R. M.; Vaez, A.; Fallin, M. D.; Hu, D.; Litonjua, A. A.; Fuemmeler, B. F.; Huen, K.; Kere, J.; Kull, I.; Munthe-Kaas, M. C.; Gehring, U.; Bustamante, M.; Saurel-Coubizolles, M. J.; Quraishi, B. M.; Ren, J.; Tost, J.; Gonzalez, J. R.; Peters, M. J.; Haberg, S. E.; Xu, Z.; van Meurs, J. B.; Gaunt, T. R.; Kerkhof, M.; Corpeleijn, E.; Feinberg, A. P.; Eng, C.; Baccarelli, A. A.; Benjamin Neelon, S. E.; Bradman, A.; Merid, S. K.; Bergstrom, A.; Herceg, Z.; Hernandez-Vargas, H.; Brunekreef, B.; Pinart, M.; Heude, B.; Ewart, S.; Yao, J.; Lemonnier, N.; Franco, O. H.; Wu, M. C.; Hofman, A.; McArdle, W.; Van der Vlies, P.; Falahi, F.; Gillman, M. W.; Barcellos, L. F.; Kumar, A.; Wickman, M.; Guerra, S.; Charles, M. A.; Holloway, J.; Auffray, C.; Tiemeier, H. W.; Smith, G. D.; Postma, D.; Hivert, M. F.; Eskenazi, B.; Vrijheid, M.; Arshad, H.; Anto, J. M.; Dehghan, A.; Karmaus, W.; Annesi-Maesano, I.; Sunyer, J.; Ghantous, A.; Pershagen, G.; Holland, N.; Murphy, S. K.; DeMeo, D. L.; Burchard, E. G.; Ladd-Acosta, C.; Snieder, H.; Nystad, W.; Koppelman, G. H.; Relton, C. L.; Jaddoe, V. W.; Wilcox, A.; Melen, E.; London, S. J. DNA Methylation in Newborns and Maternal Smoking in Pregnancy: Genome-wide Consortium Meta-analysis. Am. J. Hum. Genet. 2016, 98 (4), 680−96.
(32) Saenen, N. D.; Vrijens, K.; Janssen, B. G.; Roels, H. A.; Neven, K. Y.; Vanden Berghe, W.; Gyselaers, W.; Vanpoucke, C.; Lefebvre, W.; De Boever, P.; Nawrot, T. S. Lower Placental Leptin Promoter Methylation in Association with Fine Particulate Matter Air Pollution during Pregnancy and Placental Nitrosative Stress at Birth in the ENVIRON-AGE Cohort. Environ. Health Perspect 2017, 125 (2), 262−268.
(33) Janssen, B. G.; Byun, H. M.; Cox, B.; Gyselaers, W.; Izzi, B.; Baccarelli, A. A.; Nawrot, T. S. Variation of DNA methylation in candidate age-related targets on the mitochondrial-telomere axis in cord blood and placenta. Placenta 2014, 35 (9), 665−72.
(34) Goodrich, J. M.; Reddy, P.; Naidoo, R. N.; Asharam, K.; Batterman, S.; Dolinoy, D. C. Prenatal exposures and DNA methylation in newborns: a pilot study in Durban, South Africa. Environ. Sci. Process Impacts 2016, 18 (7), 908−17.
(35) Herbstman, J. B.; Tang, D.; Zhu, D.; Qu, L.; Sjodin, A.; Li, Z.; Camann, D.; Perera, F. P. Prenatal exposure to polycyclic aromatic hydrocarbons, benzo[a]pyrene-DNA adducts, and genomic DNA methylation in cord blood. Environ. Health Perspect 2012, 120 (5), 733−8.
(36) Perera, F.; Tang, W. Y.; Herbstman, J.; Tang, D.; Levin, L.; Miller, R.; Ho, S. M. Relation of DNA methylation of 5′-CpG island of ACSL3 to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. PLoS One 2009, 4 (2), e4488.

(37) Suila, H.; Hirvonen, T.; Ritamo, I.; Natunen, S.; Tuimala, J.; Laitinen, S.; Anderson, H.; Nystedt, J.; Rabina, J.; Valmu, L. Extracellular o-linked N-acetylglucosamine is enriched in stem cells derived from human umbilical cord blood. BioRes. Open Access 2014, 3 (2), 39−44.
(38) Lim, J. S.; Ibaseta, A.; Fischer, M. M.; Cancilla, B.; O'Young, G.; Cristea, S.; Luca, V. C.; Yang, D.; Jahchan, N. S.; Hamard, C.; Antoine, M.; Wislez, M.; Kong, C.; Cain, J.; Liu, Y. W.; Kapoun, A. M.; Garcia, K. C.; Hoey, T.; Murriel, C. L.; Sage, J. Intratumoural heterogeneity generated by Notch signalling promotes small-cell lung cancer. Nature 2017, 545 (7654), 360−364.
(39) Li, M.; Sun, Q.; Wang, X. Transcriptional landscape of human cancers. Oncotarget 2017, 8 (21), 34534−34551.
(40) Sawaguchi, S.; Varshney, S.; Ogawa, M.; Sakaidani, Y.; Yagi, H.; Takeshita, K.; Murohara, T.; Kato, K.; Sundaram, S.; Stanley, P.; Okajima, T. O-GlcNAc on NOTCH1 EGF repeats regulates ligand-induced Notch signaling and vascular development in mammals. eLife 2017, 6, e24419.
(41) Barthelery, N. J.; Manfredi, J. J. Cerebellum Development and Tumorigenesis: A p53-Centric Perspective. Trends Mol. Med. 2016, 22 (5), 404−13.
(42) Hou, L.; Zhang, X.; Tarantini, L.; Nordio, F.; Bonzini, M.; Angelici, L.; Marinelli, B.; Rizzo, G.; Cantone, L.; Apostoli, P.; Bertazzi, P. A.; Baccarelli, A. Ambient PM exposure and DNA methylation in tumor suppressor genes: a cross-sectional study. Part. Fibre Toxicol. 2011,
(43) Perera, F. P.; Li, Z.; Whyatt, R.; Hoepner, L.; Wang, S.; Camann, D.; Rauh, V. Prenatal airborne polycyclic aromatic hydrocarbon exposure and child IQ at age 5 years. Pediatrics 2009, 124 (2), e195− 202.
(44) Guxens, M.; Aguilera, I.; Ballester, F.; Estarlich, M.; Fernandez-Somoano, A.; Lertxundi, A.; Lertxundi, N.; Mendez, M. A.; Tardon, A.; Vrijheid, M.; Sunyer, J.; Project, I. Prenatal exposure to residential air pollution and infant mental development: modulation by antioxidants and detoxification factors. Environ. Health Perspect 2012, 120 (1), 144− 9.
(45) Porta, D.; Narduzzi, S.; Badaloni, C.; Bucci, S.; Cesaroni, G.; Colelli, V.; Davoli, M.; Sunyer, J.; Zirro, E.; Schwartz, J.; Forastiere, F. Air Pollution and Cognitive Development at Age 7 in a Prospective Italian Birth Cohort. Epidemiology 2016, 27 (2), 228−36.
(46) Hannon, E.; Lunnon, K.; Schalkwyk, L.; Mill, J. Interindividual methylomic variation across blood, cortex, and cerebellum: implications for epigenetic studies of neurological and neuropsychiatric phenotypes. Epigenetics 2015, 10 (11), 1024−32.
(47) Petilla Interneuron Nomenclature, G.; Ascoli, G. A.; Alonso-Nanclares, L.; Anderson, S. A.; Barrionuevo, G.; Benavides-Piccione, R.; Burkhalter, A.; Buzsaki, G.; Cauli, B.; Defelipe, J.; Fairen, A.; Feldmeyer, D.; Fishell, G.; Fregnac, Y.; Freund, T. F.; Gardner, D.; Gardner, E. P.; Goldberg, J. H.; Helmstaedter, M.; Hestrin, S.; Karube, F.; Kisvarday, Z. F.; Lambolez, B.; Lewis, D. A.; Marin, O.; Markram, H.; Munoz, A.; Packer, A.; Petersen, C. C.; Rockland, K. S.; Rossier, J.; Rudy, B.; Somogyi, P.; Staiger, J. F.; Tamas, G.; Thomson, A. M.; Toledo-Rodriguez, M.; Wang, Y.; West, D. C.; Yuste, R. Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat. Rev. Neurosci. 2008, 9 (7), 557−68.
(48) Kirmse, K.; Kummer, M.; Kovalchuk, Y.; Witte, O. W.; Garaschuk, O.; Holthoff, K. GABA depolarizes immature neurons and inhibits network activity in the neonatal neocortex in vivo. Nat. Commun. 2015, 6, 7750.
(49) Rozycka, A.; Liguz-Lecznar, M. The space where aging acts: focus on the GABAergic synapse. Aging Cell 2017, 16 (4), 634−643.
(50) Saenen, N. D.; Plusquin, M.; Bijnens, E.; Janssen, B. G.; Gyselaers, W.; Cox, B.; Fierens, F.; Molenberghs, G.; Penders, J.; Vrijens, K.; De Boever, P.; Nawrot, T. S. In Utero Fine Particle Air Pollution and Placental Expression of Genes in the Brain-Derived Neurotrophic Factor Signaling Pathway: An ENVIRONAGE Birth Cohort Study. Environ. Health Perspect 2015, 123 (8), 834−40.
(51) Vaz, S. H.; Jorgensen, T. N.; Cristovao-Ferreira, S.; Duflot, S.; Ribeiro, J. A.; Gether, U.; Sebastiao, A. M. Brain-derived neurotrophic factor (BDNF) enhances GABA transport by modulating the trafficking of GABA transporter-1 (GAT-1) from the plasma membrane of rat cortical astrocytes. J. Biol. Chem. 2011, 286 (47), 40464−76.
(52) Kim, K. H.; Kabir, E.; Kabir, S. A review on the human health impact of airborne particulate matter. Environ. Int. 2015, 74, 136−43.
(53) Flanagan, J. M.; Brook, M. N.; Orr, N.; Tomczyk, K.; Coulson, P.; Fletcher, O.; Jones, M. E.; Schoemaker, M. J.; Ashworth, A.; Swerdlow, A.; Brown, R.; Garcia-Closas, M. Temporal stability and determinants of white blood cell DNA methylation in the breakthrough generations study. Cancer Epidemiol., Biomarkers Prev. 2015, 24 (1), 221−9.
(54) Acevedo, N.; Reinius, L. E.; Vitezic, M.; Fortino, V.; Soderhall, C.; Honkanen, H.; Veijola, R.; Simell, O.; Toppari, J.; Ilonen, J.; Knip, M.; Scheynius, A.; Hyoty, H.; Greco, D.; Kere, J. Age-associated DNA methylation changes in immune genes, histone modifiers and chromatin remodeling factors within 5 years after birth in human blood leukocytes. Clin. Epigenet. 2015, 7 (1), 34.
(55) Alisch, R. S.; Barwick, B. G.; Chopra, P.; Myrick, L. K.; Satten, G. A.; Conneely, K. N.; Warren, S. T. Age-associated DNA methylation in pediatric populations. Genome Res. 2012, 22 (4), 623−32.
(56) Martino, D.; Loke, Y. J.; Gordon, L.; Ollikainen, M.; Cruickshank, M. N.; Saffery, R.; Craig, J. M. Longitudinal, genome-scale analysis of DNA methylation in twins from birth to 18 months of age reveals rapid epigenetic change in early life and pair-specific effects of discordance. Genome Biol. 2013, 14 (5), R42.
(57) Martino, D. J.; Tulic, M. K.; Gordon, L.; Hodder, M.; Richman, T. R.; Metcalfe, J.; Prescott, S. L.; Saffery, R. Evidence for age-related and individual-specific changes in DNA methylation profile of mononuclear cells during early immune development in humans. Epigenetics 2011, 6(9), 1085−94.
(58) Joubert, B. R.; Haberg, S. E.; Nilsen, R. M.; Wang, X.; Vollset, S. E.; Murphy, S. K.; Huang, Z.; Hoyo, C.; Midttun, O.; Cupul-Uicab, L. A.; Ueland, P. M.; Wu, M. C.; Nystad, W.; Bell, D. A.; Peddada, S. D.; London, S. J. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. Environ. Health Perspect 2012, 120 (10), 1425−31.
(59) Gaunt, T. R.; Shihab, H. A.; Hemani, G.; Min, J. L.; Woodward, G.; Lyttleton, O.; Zheng, J.; Duggirala, A.; McArdle, W. L.; Ho, K.; Ring, S. M.; Evans, D. M.; Davey Smith, G.; Relton, C. L. Systematic identification of genetic influences on methylation across the human life course. Genome Biol. 2016, 17, 61.
(60) Wang, D.; Liu, X.; Zhou, Y.; Xie, H.; Hong, X.; Tsai, H. J.; Wang, G.; Liu, R.; Wang, X. Individual variation and longitudinal pattern of genome-wide DNA methylation from birth to the first two years of life. Epigenetics 2012, 7 (6), 594−605.
(61) Urdinguio, R. G.; Torro, M. I.; Bayon, G. F.; Alvarez-Pitti, J.; Fernandez, A. F.; Redon, P.; Fraga, M. F.; Lurbe, E. Longitudinal study of DNA methylation during the first 5 years of life. J. Transl. Med. 2016, 14 (1), 160.

(62) Pope, C. A., 3rd; Hansen, M. L.; Long, R. W.; Nielsen, K. R.; Eatough, N. L.; Wilson, W. E.; Eatough, D. J. Ambient particulate air pollution, heart rate variability, and blood markers of inflammation in a panel of elderly subjects. Environ. Health Perspect 2004, 112 (3), 339− 45.
(63) Peters, A.; Doring, A.; Wichmann, H. E.; Koenig, W. Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 1997, 349 (9065), 1582−7.
(64) Kilinc, E.; Van Oerle, R.; Borissoff, J. I.; Oschatz, C.; Gerlofs-Nijland, M. E.; Janssen, N. A.; Cassee, F. R.; Sandstrom, T.; Renne, T.; Ten Cate, H.; Spronk, H. M. Factor XII activation is essential to sustain the procoagulant effects of particulate matter. J. Thromb. Haemostasis 2011, 9 (7), 1359−67.
(65) Riediker, M.; Cascio, W. E.; Griggs, T. R.; Herbst, M. C.; Bromberg, P. A.; Neas, L.; Williams, R. W.; Devlin, R. B. Particulate matter exposure in cars is associated with cardiovascular effects in healthy young men. Am. J. Respir. Crit. Care Med. 2004, 169 (8), 934−40.
(66) Kannan, S.; Misra, D. P.; Dvonch, J. T.; Krishnakumar, A. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition. Environ. Health Perspect 2006, 114 (11), 1636−42.
(67) Breton, C. V.; Marsit, C. J.; Faustman, E.; Nadeau, K.; Goodrich, J. M.; Dolinoy, D. C.; Herbstman, J.; Holland, N.; LaSalle, J. M.; Schmidt, R.; Yousefi, P.; Perera, F.; Joubert, B. R.; Wiemels, J.; Taylor, M.; Yang, I. V.; Chen, R.; Hew, K. M.; Freeland, D. M.; Miller, R.; Murphy, S. K. Small-Magnitude Effect Sizes in Epigenetic End Points are Important in Children's Environmental Health Studies: The Children's Environ-mental Health and Disease Prevention Research Center's Epigenetics Working Group. Environ. Health Perspect 2017, 125 (4), 511−526.
(68) Eeftens, M.; Beelen, R.; Fischer, P.; Brunekreef, B.; Meliefste, K.; Hoek, G. Stability of measured and modelled spatial contrasts in NO(2) over time. Occup. Environ. Med. 2011, 68 (10), 765−70.